BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

ELSEVIER

Immunopharmacology 43 (1999) 169–177

Immunopharmacology

www.elsevier.com/locate/immpharm

Small molecule antagonists of the bradykinin B1 receptor

Robert A. Horlick *, Michael H. Ohlmeyer, Ilana L. Stroke, Barbara Strohl, Gonghua Pan, Adriane E. Schilling, Vidyadhar Paradkar, Jorge G. Quintero, Ming You, Christopher Riviello, Megan B. Thorn, Bassam Damaj, V. Danial Fitzpatrick, Roland E. Dolle, Maria L. Webb, John J. Baldwin, Nolan H. Sigal

Pharmacopeia, CN5350, Princeton, NJ 08543-5350, USA

Accepted 3 June 1999

Abstract

Screening Pharmacopeia's encoded combinatorial libraries has led to the identification of potent, selective, competitive antagonists at the bradykinin B1 receptor. Libraries were screened using a displacement assay of [^{3}H]-des-Arg^{10}-kallidin ([^{3}H]-dAK) at IMR-90 cells expressing an endogenous human B1 receptor (B_{max} = 20,000 receptors/cell, $K_D = 0.5 \pm 0.1$ nM) or against membranes from 293E cells expressing a recombinant human B1 receptor (B_{max} = 8,000 receptors/cell, $K_D = 0.5 \pm 0.3$ nM). Compound PS020990, an optimized, representative member from the class of compounds, inhibits specific binding of ^{3}H-dAK at IMR-90 cells with a K_i of 6 ± 1 nM. The compound inhibits dAK-induced phosphatidyl inositol turnover ($K_{Bapp} = 0.4 \pm 0.2$ nM) and calcium mobilization ($K_{Bapp} = 17 \pm 2$ nM) in IMR-90 cells. Compounds from the lead series are inactive at the B2 receptor and are > 1000-fold specific for B1 vs. a variety of other receptors, ion channels and enzymes. PS020990 and other related chemotypes therefore offer an excellent opportunity to explore further the role of B1 receptors in disease models and represent a potential therapeutic avenue.

Keywords: Combinatorial chemistry; Chemical libraries; Receptor binding; Receptor signaling; High-throughput screening; GPCR

1. Introduction

The kinins, potent mediators of inflammation and algesia, are known to exert their actions through binding to two receptor subtypes (Regoli and Barabé, 1980). The subtypes differ in their affinities for various natural and synthetic peptide ligands as well as in their regional and temporal expression. For example, the B1 receptor is not normally present in healthy tissues but rather its expression is induced by immunopathology or following injury, whereas the B2 receptor is widely and constitutively expressed (Dray and Perkins, 1993; Davis and Perkins, 1994). The B1 receptor is thought to play a role in chronic inflammation, hyperalgesia and septic shock, whereas

Abbreviations: dAK, Des-Arg^{10}-kallidin or lys^0-des-Arg^9-bradykinin; B1, Bradykinin receptor subtype 1; B2, Bradykinin receptor subtype 2; BK, Bradykinin; EGF, Epidermal growth factor; FBS, Fetal bovine serum; IL1β, Interleukin 1β; K_{Bapp}, K_B apparent; LPS, Bacterial lipopolysaccharide; PBS, Phosphate-buffered saline; PI, Phosphatidyl inositol; PITO, PI turnover

* Corresponding author

0162-3109/99/$ - see front matter
PII: S0162-3109(99)00130-7

evidence suggests that the B2 receptor acts as a mediator of acute inflammatory and algesic responses (Menke et al., 1994; Marceau et al., 1997; for review of B1, see Marceau, 1995). Several animal models suggest that antagonism at the B1 receptor would represent a very attractive target for antinociceptive therapy. While several peptide and non-peptide antagonists at the B2 receptor are available and have been studied, only peptide antagonists at the B1 receptor have been reported to date.

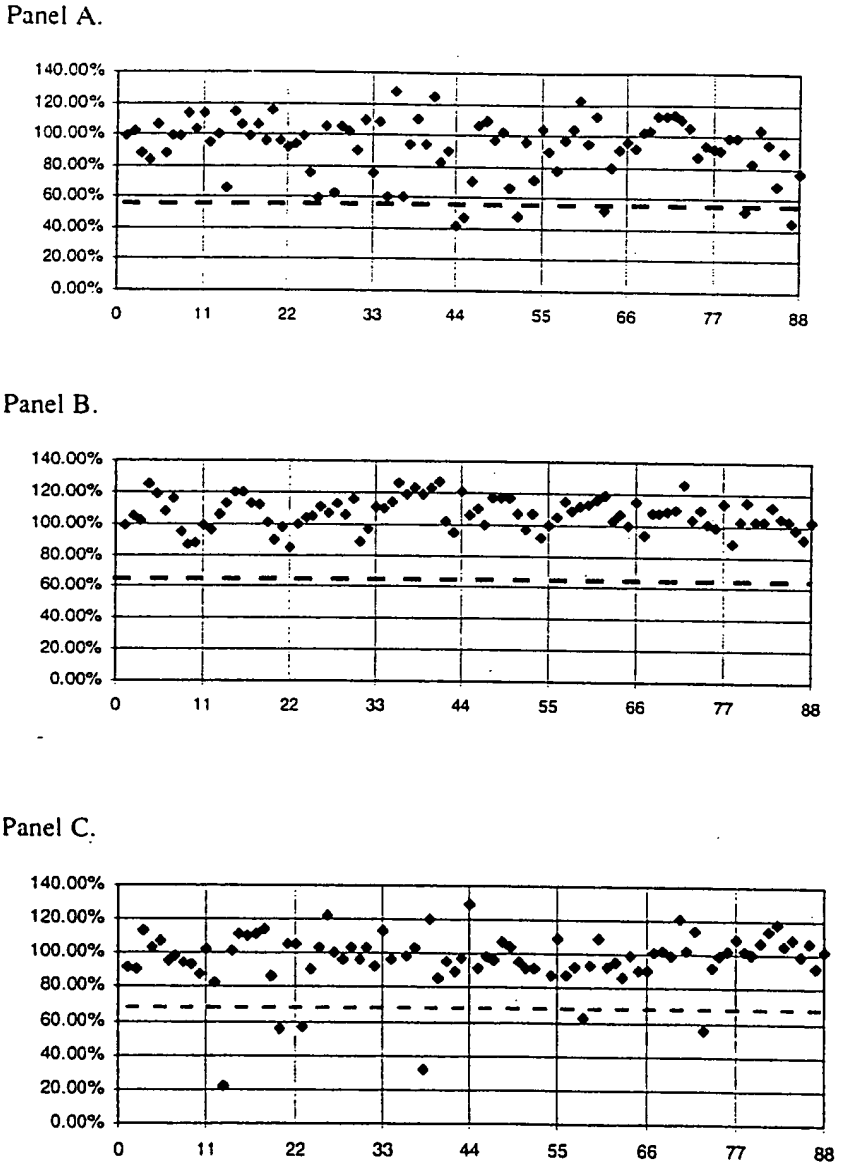


Fig. 1. Ligand displacement activity profile from an active and inactive sublibrary. Panel A: Profile of a sublibrary containing numerous active compounds; 20 compounds per well. Panel B: Profile from a typical inactive sublibrary; 20 compounds per well. Panel C: The rearray plate contains a single compound per well. Numbers on the abscissa refer to microtiter dish well number. Numbers on the ordinate refer to percent activity as compared to the no compound control wells. Dashed line indicates 3 standard deviations from the mean and represents the threshold for distinguishing active compounds.

Using ECLiPS™(Encoded Combinatorial Library on Polymeric Support), a proprietary tagging technology, Pharmacopeia has generated large combinatorial chemical libraries, now consisting of 3.9 million diverse, easily identifiable, small molecules (Ohlmeyer et al., 1993; Chabala, 1995; Appell et al., 1996). A high-throughput screening assay was developed to search Pharmacopeia's chemical libraries for small molecule antagonists at the human B1 receptor. Several active chemical compounds were identified in one library that generated a useful initial structure–activity relationship (SAR). The SAR information was used to generate additional chemical entities with improved potency. Described below are the properties of one such novel small-molecule, non-peptide antagonist at the human B1 receptor.

2. Materials and methods

2.1. Cell lines

2.1.1. Natively expressing human B1 cell line

The IMR-90 cell line, a human lung fibroblast derived line that can be induced to express its endogenous B1 receptor (Goldstein and Wall, 1984; Menke et al., 1994), was obtained from the American Type Culture Collection (Rockville, MD). Cells were cultured in MEM with Earle's BSS (Life Technologies) supplemented with 10% FBS, 1 mM sodium pyruvate, non-essential amino acids and 5 mM glutamine and were maintained for no more than 34 passages. To induce expression of the endogenous B1 receptor, IMR-90 cells were treated for 4 h at 37°C in humidified 5% CO_2 atmosphere with 0.2 ng/ml interleukin 1β (IL1β) in DMEM media with pyruvate supplemented with 10% FBS, non-essential amino acids and 5 mM L-glutamine (induction media) (Goldstein and Wall, 1984; Menke et al., 1994).

2.1.2. Construction of a transfected human B1 cell line

Since the entire coding sequence for the human B1 receptor is contained within a single exon (Marceau et al., 1997), the coding region was PCR amplified directly from human genomic DNA (Promega, Madison, WI). The resulting clone was identical to the variant reported by Aramori et al. (1997). A Kozak consensus sequence (Kozak, 1986) was incorporated into the 5′ oligonucleotide and the resulting fragment was cloned into an episomal vector, pE3 (Horlick et al., 1997). The B1-expressing vector, pE3-hB1, was stably transfected into 293EBNA cells (Invitrogen, Carlsbad, CA) using the calcium phosphate method of Sambrook et al. (1989) to generate the cell line, 293E-hB1. Cells were maintained in DMEM medium supplemented with 10% FBS, 5 mM Glutamax (Life Technologies, Gaithersburg, MD), 100 U/ml penicillin, 100 μg/ml streptomycin and 250 μg/ml hygromycin B. In contrast to IMR-90 cells, 293E-hB1 cells did not require induction to express the B1 receptor.

2.2. Screening and receptor binding strategy

Pharmacopeia ECLiPS™ Libraries were screened in binding displacement assays as described in Appell et al. (1996) using induced IMR-90 cells or 293E-hB1 membranes. Resynthesized compounds were sonicated in 100% DMSO for 10 min at room temperature and diluted to 1 mM or 0.1 mM in 100% DMSO, as appropriate. Binding assays were performed in assay buffer (M199 media (Sigma), 25 mM HEPES buffer, 0.2% BSA, 1 μM phosphorami-

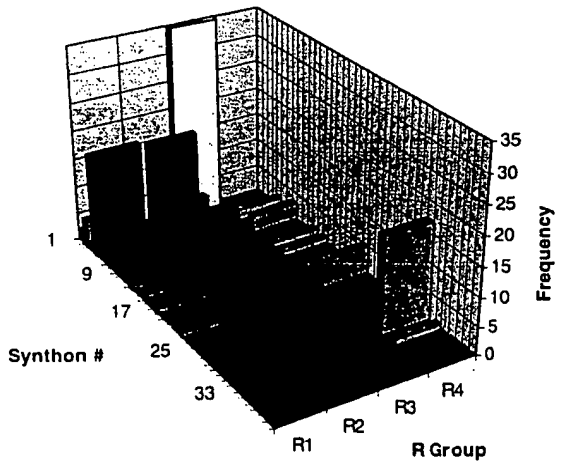
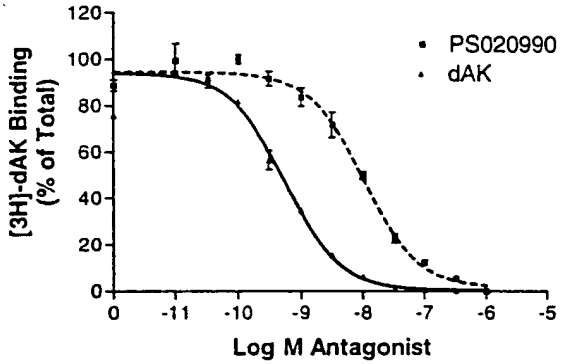


Fig. 2. Synthon frequency table. The core chemical scaffold in the active library contains four positions of variability with 31, 31, 4 and 38 possible substituents or "synthons" at each of the four positions, respectively. Summarized are the results for 35 decoded compounds.

don, 1 μM Captopril, 2 μM DTT, and 3 μM amastatin) in the presence of 0.75 nM [^{3}H]-dAK, antagonist at the appropriate concentration, 4% DMSO, and 50,000 cells (IMR-90) or 100,000 cell equivalents (293E-B1) in 96-well microtiter dish format. Binding reactions were allowed to proceed for 1.5 h at room temperature and were terminated by addition of 150 μl ice-cold PBS. Reactions were transferred to glass fiber filter plate that was blocked with polyethylimine and washed 3 × with ice cold PBS. 40 ul per well of scintillation fluid was added and filters were counted for 10 min per well.

Panel A.



Panel B.

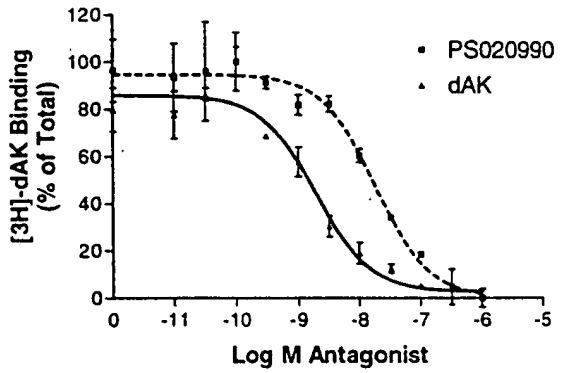


Fig. 3. Inhibition of [^{3}H]-dKD binding to B1 receptors by PS020990 in IMR-90 and 293E-B1 cells. Binding was performed with 0.75 nM of [^{3}H]-dAK and varying amounts of dAK or PS020990. Shown are graphs taken from a single experiment representative of three independent assays. Panel A: IMR-90 cells; Panel B: 293E-B1.

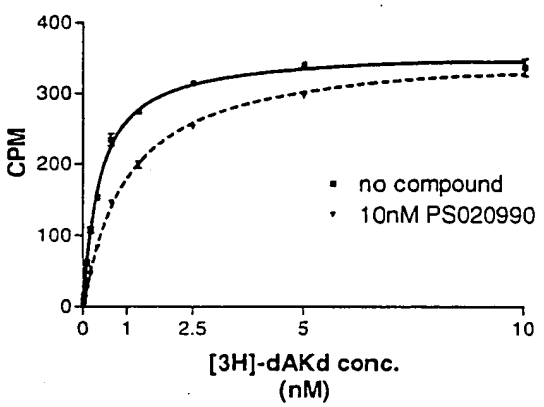


Fig. 4. Competition of [^{3}H]-dAK saturation binding by PS020990 in IMR-90 cells. Saturation binding was performed in the presence or absence of 10 nM PS020990.

2.3. Calcium mobilization assays

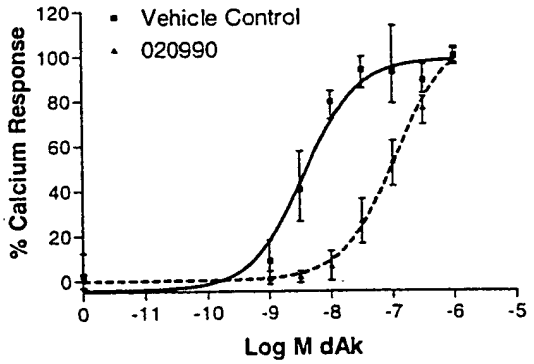
Calcium mobilization assays were performed with modifications of the procedure of Tsien et al. (1982). IMR-90 cells were treated with IL1β to induce B1 receptors, trypsinized from flask, counted, and resuspended at 3×10^6 cells/ml in assay buffer (described above) containing 1 mM probenecid. Fluo3 (in DMSO containing 10% Pluronic F127) was added to the culture at a final concentration of 9 ng/ml and cells were gently rocked for 1 h in the dark. Cells were washed twice with assay buffer and resuspended at 6×10^6 cell/ml in assay buffer. To each well, 25 μl of 2 × -concentrated agonist and 2 μl of 25 × -concentrated antagonist, or buffer alone, was added. Reactions were initiated by addition of 25 μl of the cell suspension and plate was read immediately on a SpeedReader (33 frames, 500 ms per frame, 1 s delay between frames; custom made by SAIC, San Diego, CA). Maximum and minimum calcium values were calculated by addition of 10 μl of 10% Triton X-100 or 10 μl of 500 mM EGTA per well, respectively, shaking for 3 min, and reading a single, still frame.

2.4. PI turnover assays

IMR-90 cells at 2.5×10^5 cells in 1.5 ml culture media were plated per well in a 6-well microtiter dish and typically 2–4 uCi of [^{3}H]-myoinositol (New

England Nuclear) was added per well. Cells were incubated at 37°C in humidified 5% CO_2 for 36–50 h. Culture media was replaced with induction media 4 h prior to assay to induce B1 expression. Immediately preceding the assay, induction media was removed and assay buffer supplemented with 20 mM LiCl was added to cells. Test compound or buffer was added and cells were incubated at 37°C for 10–15 min. dAK was subsequently added and cells were incubated a further 30–45 min at 37°C. Reactions were terminated by removal of media and addition of 2 ml of boiling 2 mM EDTA (pH 5–6). Cooled lysate was loaded on a 1 ml AG1-X8 Dowex resin (BioRad) that had been prewashed with formate buffer (4 M ammonium formate, 200 mM formic acid) and pre-equilibrated with 170 mM aqueous NH_4OH. Loaded columns were rinsed first with 170 mM aqueous NH_4OH, then with (40 mM ammonium formate, 0.1 M formic acid), and radioactive inositol phosphates were eluted with 1 ml (4 M ammonium formate, 0.2 M formic acid). Samples were counted in the presence of 2 ml scintillation fluid.

Panel A.



Panel B.

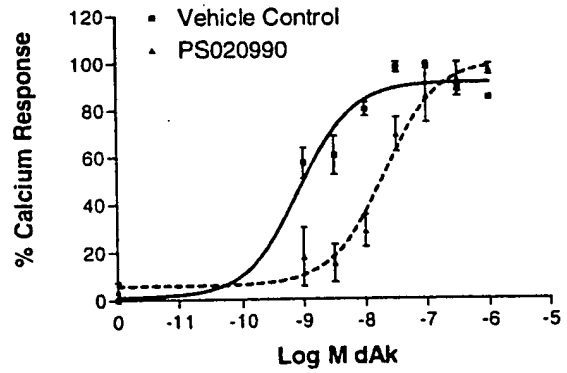


Fig. 5. Effect of PS020990 on Ca flux in IMR90 and transfected 293E cells mediated by dAK at the B1 receptor. The graph shown is a composite of data from three independent experiments. Panel A: IMR90 cells: Panel B: 293E-b1 cells.

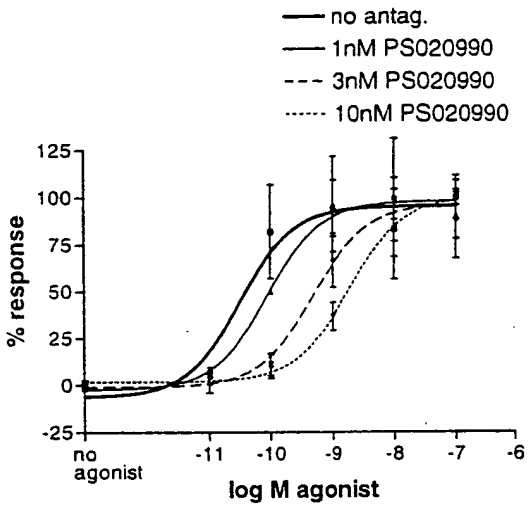


Fig. 6. Effect of PS020990 on PI turnover in IMR90 cells mediated by dAK at the B1 receptor. Data shown are representative of three independent assays.

3. Results

3.1. Identification of compounds active at the B1 receptor

A high-throughput assay to measure the binding of [^{3}H]-dAK to B1 receptors on IMR-90 cells was established in 96-well microtiter dish format. In a screen of a 150,000 member chemical library arrayed at 20 compounds per well, several wells showed activity in inhibiting ligand binding. Inhibition of [^{3}H]-dAK binding appeared in several sublibraries (sections of the library defined by the synthon added during the last step of combinatorial synthesis, Fig. 1a–b). Rearray of positive wells to single compounds per well led to confirmation of the primary

Table 1
Specificity of compound PS309799

Receptor	% Inhibition at 10 μM PS309799
Neurotransmitter-related	
Adenosine	12
Adrenergic, alpha 1, non-selective	3
Adrenergic, alpha 2, non-selective	15
Adrenergic, beta, non-selective	0
Dopamine transporter	27
Dopamine, non-selective	14
GABA A, agonist site	0
GABA A, benzodiazepine, central	0
Glutamate, AMPA site	0
Glutamate, kainate site	0
Glutamate, NMDA agonist site	0
Glycine, strychnine sensitive	0
Histamine H1	0
Histamine H2	12
Muscarinic, M2	17
Muscarinic, non-selective, central	13
Muscarinic, non-selective, peripheral	5
Nicotinic, a-bungarotoxin insensitive	0
Norepinephrine transporter	5
Opiate, non-selective	3
Serotonin transporter	13
Serotonin, non-selective	0
Sigma, non-selective	0
Steroids	
Estrogen	13
Testosterone	0
Ion channels	
Calcium channel, type L	21
Calcium channel, type N	0
K^+ channel, ATP-sensitive	2
K^+ channel, Ca-activated, VI	2
K^+ channel, Ca-activated, VS	9
Sodium channel, site 2	13
Second messengers	
NOS, neuronal	0
Prostaglandins	
Leukotriene B4, LTB4	4
Leukotriene D4, LTD4	38
Thromboxane A2	6
Growth factors / hormones	
Corticotropin releasing factor	32
Platelet activating factor	0
Thyrotropin releasing hormone	0
Brain / gut peptides	
Angiotensin II, AT1	0
Angiotensin II, AT2	0
Bradykinin B2	12
CGRP, central	0
Cholecystokinin, CCK1	0
Cholecystokinin, CCK2	1
Endothelin, ET-A	16
Endothelin, ET-B	20
Galanin	10
Neurokinin, NK1	0
Neurokinin, NK2	0
Neurokinin, NK3	0
Vasoactive intestinal peptide, non-selective	18
Vasopressin 1	15
Enzymes	
Acetylcholinesterase	0
Choline acetyltransferase	0
Glutamic acid decarboxylase	4
Monoamine oxidase B, MAO-B	20

screen activity (Fig. 1c). These structures were decoded and several compounds were resynthesized for assessment in ligand displacement assays. The initial screening hits in the chemical series had affinities for the B1 receptor in the range of 200–400 nM. Analysis of the active substituents in the four combinatorial positions of variation in the structures indicated a distinct synthon preference (Fig. 2). For instance, of 31 possible substituents at position 1, synthon 4 was found in 18 out of 35 decoded structures. Likewise, at position 2, of 31 possibilities synthon 7 was found 19 times; whereas synthon 1 was the only chemical moiety identified at position 3 in all 35 structures. Of 38 sublibraries representing position 4, sublibrary 31 was the most active, providing nearly half of the decoded molecules. The characterization of synthon preferences and variations permitted a good initial understanding of a structure–activity relationship for subsequent synthesis of analogs. Through a combination of screening parallel synthesis chemical libraries and traditional medicinal chemistry efforts, a significant number of compounds were generated with affinity at the human B1 receptor of 1–10 nM. One such analog, PS020990, inhibited binding of [^{3}H]-dAK [$K_D = 0.3 \pm 0.07$ nM, (24)] to cytokine-induced IMR-90 and 293E-hB1 cells, with K_i values of 6 ± 1 nM (9) (Fig. 3, panel A) and 19 ± 14 nM (3) (Fig. 3, panel B), respectively. Binding inhibition was complete with reduction of [^{3}H]-dAK to non-specific binding levels and slopes of the inhibition curves were not significantly different from unity.

When saturation binding of [^{3}H]-dAK is performed in the presence of 10 nM PS020990, there is a rightward shift in the K_D value without any change in the B_{max} consistent with a competitive mechanism of binding (Fig. 4).

3.2. Characterization of PS020990 in signal transduction assays

The B1 receptor is known to transduce intracellular signals via coupling with the G protein, Gq, and activation of phospholipase C that can be observed as a transient flux of intracellular calcium and as an increase in phosphatidyl inositol (PI) turnover (PITO) (Marsh and Hill, 1994; Butt et al., 1995; Austin et al., 1997). To address the question of whether PS020990 is a bona fide antagonist, the ability of the compound to inhibit the mobilization of intracellular calcium and accumulation of PI was measured. In both IMR-90 cells (expressing endogenous B1 receptors) as well as stably transfected 293E-hB1 cells, PS020990 inhibited dAK-induced intracellular calcium mobilization. dAK causes a mobilization of calcium with an EC50 of 4.2 ± 0.5 nM (11). In the presence of PS020990, the agonist–response curves were shifted rightward with slopes near unity, and without reduction in maximal agonist effects. The K_{Bapp} values in IMR-90 and 293E-hB1 cells were 17 ± 2 nM (4) and 45 ± 11 nM (4), respectively (Fig. 5). These Ca^{2+} K_{Bapp} values are consistent with K_i values from binding. Similarly, increasing concentrations of the compound inhibited dAK-induced phosphatidyl inositol turnover in IMR-90 cells as measured by a series of parallel, rightward shifted response curves (dAK EC50 = 0.17 ± 0.05 nM (12); PS020990 K_{Bapp} = 0.4 ± 0.2 nM (2); Fig. 6) providing further evidence for a competitive mechanism of binding. PS020990 has no intrinsic agonist activity as evidenced by failure to elicit calcium flux or PI induction at a concentration of 1 μM (data not shown).

3.3. Specificity of binding of the lead series

The ability of several molecules in the same chemical series as PS020990 to inhibit binding of [^{3}H]-bradykinin ([^{3}H]-BK) at the B2 receptor was assessed. Members of the series fail to inhibit [^{3}H]-BK binding (Table 1) or BK-induced calcium mobilization (data not shown) at 10 μM concentration. Compound PS309799, a closely related chemotype to PS020990, has an affinity at the B1 receptor of 3 ± 1 nM and was evaluated for specificity by assessing its activity in 57 different receptor, ion channel and enzyme-based assays. At a concentration of 10 μM, PS309799 had little or no activity in any of the assays (Table 1). These data are consistent with the suggestion that PS309799 and related compounds are highly selective for the B1 receptor subtype.

4. Discussion

PS020990 is a member of a class of molecules that may be considered as being composed of four domains (corresponding to the combinatorial synthetic steps from which it was derived), the central element of which is a bi-heteroaryl core. Potent members of the class contain a range of hydrogen bond donating and accepting functionality, for example, PS020990 has three hydrogen bond donor and five hydrogen bond acceptor sites. PS020990, one of the more lipophilic examples, has a molecular weight of 611 Da, but other members of the series with K_i < 20 nM have lower logP and range in molecular weight from 457 to 612 Da. The active molecules are in general drug like and they are devoid of functionality often associated with toxicity, for example nitro, aniline, active halogen or Michael acceptors and they do not contain metabolically labile moieties such as esters, sulfhydryl or phenols.

The data reported here demonstrate that PS020990 is a potent, selective, competitive inhibitor of human B1 receptors expressed in cytokine-induced, natively expressing cells, as well as in a recombinantly engineered, constitutively expressing cell line. The compound potently inhibits dAK-induced mobilization of intracellular calcium and accumulation of PI, consistent with its action as an antagonist. There are no significant differences in the potency of compound PS020990 in binding nor in inhibition of calcium mobilization, between the inducible, native B1 receptor in IMR-90 cells as compared to the constitutively expressed, recombinant B1 receptor in 293E cells.

This is consistent with the findings of Bastian et al. (1997) who find no differences in affinity of various BK and dAK related peptides at the native vs. recombinantly expressed B1 receptors.

It is interesting to note differences among the various measures of compound potency. The K_I of PS020990 at the B1 receptor is 6 nM, for inhibition of calcium mobilization the K_{Bapp} is 17 nM, and for inhibition of PITO the K_{Bapp} is 0.4 nM. While the binding inhibition constant and K_{Bapp} calcium flux inhibition figures are consistent based on receptor occupancy considerations, the potency of the compound in inhibiting accumulation of PI is greater than expected. The molecular basis for this difference is unknown but could be due to the existence of reserve or "spare" receptors, to coupling via alternative G protein species, or to another, as yet unelucidated mechanism.

Several B1 specific peptide antagonists, especially des-Arg^{10}, Leu^{9}-kallidin, have been widely available for several years and have been used to characterize the role of B1 receptors in various physiopathologies. However, while des-Arg^{10}, Leu^{9}-kallidin and related prototypical B1 peptides do indeed function as antagonists at the human B1 receptor, they have been found to exhibit significant partial agonist activity at the canine and murine B1 receptor (MacNeil et al., 1997; Regoli et al., 1998). The agonist activity renders these peptides less useful as tools in characterizing the role of B1 receptor in animal models of pain and inflammation. Only very recently have metabolically stable, B1 receptor peptide antagonists devoid of agonist activity been described (Regoli et al., 1998). To our knowledge, the work described in this report represents the first description of a potent, selective, non-peptide antagonist for the human bradykinin B1 receptor. We anticipate that PS020990 and its related analogs will be useful in assessing the role of the B1 receptor in various immunopathological conditions.

References

Appell, K.C., Chung, T.D.Y., Ohlmeyer, M.J.H., Sigal, N.H., Baldwin, J.J., Chelsky, D., 1996. Biological screening of a large combinatorial library. J. Biomol. Screening 1, 27–31.

Aramori, I., Zenkoh, J., Morikawa, N., O'Donnell, N., Asano, M., Nakamura, K., Iwami, M., Kojo, H., Notsu, Y., 1997. Novel subtype-selective nonpeptide bradykinin receptor antagonists FR167344 and FR173657. Mol. Pharmacol. 51, 171–176.

Austin, C.E., Faussner, A., Robinson, H.E., Chakravarty, S., Kyle, D.J., Bathon, J.M., Proud, D., 1997. Stable expression of the human kinin B1 receptor in Chinese hamster ovary cells. Characterization of ligand binding and effector pathways. J. Biol. Chem. 272, 11420–11425.

Bastian, S., Loillier, B., Paquet, J.L., Pruneau, D., 1997. Stable expression of human kinin B1 receptor in 293 cells: pharmacological and functional characterization. Br. J. Pharmacol. 122, 393–399.

Butt, S.K., Dawson, L.G., Hall, J.M., 1995. Bradykinin B1 receptors in the rabbit urinary bladder: induction of responses, smooth muscle contraction, and phosphatidylinositol hydrolysis. Br. J. Pharmacol. 114, 612–617.

Chabala, J.C., 1995. Solid-phase combinatorial chemistry and novel tagging methods for identifying leads. Curr. Opin. Biotechnol. 6, 632–639.

Davis, A.J., Perkins, M.N., 1994. Induction of B1 receptors in vivo in a model of persistent inflammatory mechanical hyperalgesia in the rat. Neuropharmacology 33, 127–133.

Dray, A., Perkins, M., 1993. Bradykinin and inflammatory pain. Trends Neurosci. 16, 99–104.

Goldstein, R.H., Wall, M., 1984. Activation of protein formation and cell division by bradykinin and des-Arg9-bradykinin. J. Biol. Chem. 259, 9263–9268.

Horlick, R.A., Sperle, K., Breth, L.A., Reid, C.C., Shen, E.S., Robbins, A.K., Cooke, G.M., Largent, B.L., 1997. Rapid generation of stable cell lines expressing corticotropin releasing hormone receptor for drug discovery. Prot. Exp. Purific. 9, 301–308.

Kozak, M., 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell 44, 283–292.

MacNeil, T., Feighner, S., Hreniuk, D.L., Hess, J.F., Van der Ploeg, L.H., 1997. Partial agonists and full antagonists at the human and murine bradykinin B1 receptors. Can. J. Physiol. Pharmacol. 75, 735–740.

Marceau, F., 1995. Kinin B1 receptors: a review. Immunopharmacology 30, 1–26.

Marceau, F., Larrivée, J.-F., Saint-Jacques, E., Bachvarov, D.R., 1997. The kinin B1 receptor: an inducible G protein coupled receptor. Can. J. Physiol. Pharmacol. 75, 725–730.

Marsh, K.A., Hill, S.J., 1994. Des-Arg^{9}-bradykinin-induced increases in intracellular calcium ion concentration in single bovine tracheal smooth muscle cells. Br. J. Pharmacol. 112, 934–938.

Menke, J.G., Borkowski, J.A., Fierilo, K.K., MacNeil, T., Derrick, A.W., Schneck, K.A., Ransom, R.W., Strader, C.D., Linemeyer, D.L., Hess, F.J., 1994. Expression cloning of a human B1 bradykinin receptor. J. Biol. Chem. 269, 21583–21586.

Ohlmeyer, M.H., Swanson, R.N., Dillard, L.W., Reader, J.C., Asouline, G., Kobayashi, R., Wigler, M., Still, W.C., 1993. Complex synthetic chemical libraries indexed with molecular tags. Proc. Natl. Acad. Sci. U.S.A. 90, 10922–10926.

Regoli, D., Barabé, J., 1980. Pharmacology of bradykinin and related kinins. Pharmacol. Rev. 32, 1–14.

Regoli, D., Nsa Allogho, S., Rizzi, A., Gobeil, F.J., 1998. Bradykinin receptors and their antagonists. Eur. J. Pharmacol. 348, 1–10.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Tsien, R.Y., Pozzan, T., Rink, T.J., 1982. Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new, intracellularly trapped fluorescent indicator. J. Cell. Biol. 94, 325–334.